CELL MOVEMENT, RATE OF REGENERATION, AND THE AXIAL GRADIENT IN TUBULARIA [1]

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One of the great unifying concepts in the field of development has been that of the axial gradient. As Child envisions the development of polarity in an organism, there is a quantitative gradient in metabolic rate which marks the primary axis, this gradient being responsible for the establishment of qualitative differences along the axis during the course of development (Child, 1941). Such gradients have been demonstrated in a wide variety of plants and animals, among which is the extensively investigated hydroid, *Tubularia*. In this animal the axial gradient is manifested physiologically by a decreasing rate of hydranth regeneration as one proceeds from distal (apical) toward proximal (basal) levels of the hydrocaulus or "stem." This phenomenon was first observed by Loeb (1892), who did not consider the differences in rate to be significant, but confirmation by a host of other workers (Driesch, 1899; Morgan, 1905; Child, 1907a; Hyman, 1920; Barth, 1938, 1940; Spratt, unpublished) leaves no doubt concerning its reality.

During a recent investigation of the mechanism of physiological dominance in *Tubularia* (Steinberg, 1954) it became necessary to measure the rates of regeneration of distal and proximal ends of stems ligated in the middle. In such stems the distal end regenerates considerably faster than the proximal end (Morgan and Stevens, 1904; Peebles, 1931; Barth, 1938). It was observed that once the primordium of the regenerating hydranth was established as a thickened zone near the cut end, differentiation seemed to proceed at about the same rate at both the distal and the proximal ends. The increased time for proximal regeneration apparently was due to a delay in the establishment of the primordium rather than to a slower rate of its differentiation into a fully formed hydranth. Since the experiment was set up with another purpose in mind, the measurements necessary to test the validity of this observation were not made, but other considerations seemed to lend weight to this view.

The axial gradient in *Tubularia* is manifested not only by differences in rate of regeneration, but also by a gradual diminution in size of the primordium formed as pieces are taken from increasingly basal regions of the stem (Driesch, 1899; Hyman, 1926; Barth, 1938). There is also a direct correlation between the length of a piece and the size of the primordium which it develops (Driesch, 1899; Child, 1907b; Peebles, 1931; Spratt, unpublished), contrary to the results of Hyman (1926), longer pieces giving rise to larger primordia. It was found by Peebles (1931) that regeneration could be delayed and the size of the as yet unformed

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primordium of a long stem reduced by removing a large piece from the proximal end of the stem only if the piece was removed before 13 hours of the regeneration period had elapsed. These observations were confirmed by the author (Steinberg, 1954), in whose material, however, a period of 8 hours sufficed to produce the effects obtained by Peebles only after 13 hours. It was shown in addition that the period preceding the appearance of the primordium is one during which the tissue shifts toward the distal end of the perisarc, providing additional cells for incorporation into the primordium, and that rate of regeneration and the size of the regenerant are both functions of the number of cells available. By these experiments it is shown that both rate of regeneration and the size of the regenerant are fixed before the primordium appears. Certain factors, such as the tissue movement, which affect both of these parameters of regeneration, appear to operate exclusively before primordium formation. The evidence presented above applies to systemic factors which affect the rate of regeneration and the size of the primordium at a given level of the stem. It does not, however, exclude the possibility that the rate of differentiation of the primordium into a completed hydranth may vary from level to level or as a function of a large number of other natural variables affecting regeneration rate.

Rate of regeneration in *Tubularia* varies as a function of the following systemic parameters: length of the piece, width of the piece (Hyman, 1926), apico-basal level from which the piece is taken, and distal versus proximal regeneration site on the piece. These variables were chosen for investigation in the present study, the purpose of which was to ascertain whether the differences in rate of regeneration which exist between long versus short stems, narrow versus wide stems, the distal versus the proximal ends of ligated stems, and finally apical versus basal stem levels all lie in the period of tissue movement, or whether under the various conditions mentioned the differentiation process itself proceeds at different rates.

Materials and Methods

Straight, clean, unbranched stems from freshly collected colonies of *Tubularia crocea* were used exclusively. The proximal cut was always made slightly oblique in order to distinguish between distal and proximal ends. Regenerating stems were kept individually in labelled stender dishes of two-inch outside diameter containing ⅜ of an inch of sea water and maintained at a fairly constant temperature on a sea water table over which fresh sea water was constantly flowing. The time of cutting of each stem was noted, and at intervals of a few hours until regeneration in all groups was complete, each stem was inspected and its regeneration stage and the time recorded. To maintain objectivity, the previous history of a stem was never consulted in making a new recording of its regeneration stage.

The animals were divided into 5 pairs of groups as follows, with 20 stems in each group.

I (20° C.)
- Group A. 8-mm. stems with the distal end taken two mm. below the hydranth.
- Group B. 8-mm. stems with the distal end taken 30 mm. below the hydranth, and from the same stems as Group A.

II (16.5–18° C.)	Group C. 2-mm. stems with the distal end taken two mm. below the hydranth. Group D. 15-mm. stems taken just below the 2-mm. stems of Group C.
III (16.5–18° C.)	Group E. 5-mm. stems of very small diameter, taken two mm. below the hydranth. Group F. 5-mm. stems of very large diameter, taken two mm. below the hydranth and from the same colony as Group E.
IV (16.5–18° C.)	Group G. The distal ends of 10-mm. stems taken two mm. below the hydranth and ligated in the middle with nylon thread. Group H. The proximal ends of the stems in Group G.
V (19–20° C.)	Group I. The same as Group G, but taken from a different colony. Group J. The proximal ends of the stems in Group I.

After all of the stems had regenerated, the period of regeneration of each stem was divided into two parts: (1) the length of time between the cutting of the stem and the appearance of the thickening which is the primordium of the regenerating hydranth (= movement time), and (2) the length of time between the appearance of the thickening and the appearance of the constriction which marks the base of the regenerated hydranth (= differentiation time). (For drawings of the stages see Steinberg, 1954.) Since the object of the experiment is to distinguish between the time required for movement and the time required for differentiation, the onset of the "thickened" stage was chosen as the division between the two periods because (1) the only known process preceding it (except for the breakdown, near the two cut ends, of the endodermal ridges which traverse the length of the stem) is the tissue movement, and (2) Davidson and Berrill (1948) have shown that this is the stage at which one finds the first evidence of determination by the test of isolation of parts of the thickening, followed by self-differentiation. It should be mentioned that determination here is only with respect to the replacement of missing proximal structures. Missing distal structures are always replaced in *Tubularia,* even by the adult hydranth.

Experimental Results

1. The basis of the axial gradient and differences in rate of regeneration

The results of the experiments are summarized in Table I. The experiment (II) in which short and long pieces are compared is not satisfactory because 14 of the 20 two-mm. stems which comprised Group C regenerated as partial bipolar forms. In these forms, common in short pieces, the entire tissue of the piece transforms into a pair of hydranths fused at their bases. Such bipolars lack proximal tentacles and in some cases gonophores as well. It is impossible to get regeneration rates in such stems because the constriction which marks the base of a regenerated hydranth and the formation of which is used as the end point of regeneration never appears. As Table I shows, no significant differences were

found, either in the movement or the differentiation phase, between groups C and D, probably because of the small number of cases in Group C. However, in every other pair of experiments there is a large difference in movement time between the two groups of the pair, the group regenerating more slowly always being the one in which the tissue movement occupies more time. This is not the case with respect to differentiation, however. In experiments III, IV and V the differentiation time of both groups of the pair is the same. Experiment I presents a different picture. Group B differentiated *more rapidly* than Group A. This is indeed remarkable, since B is the more slowly regenerating group of the pair,

TABLE I

Mean time required, with the standard error of the mean, for the entire regeneration process and for its two component parts, tissue movement and differentiation, in the various paired experimental groups; p indicates the probability that the difference between the two groups of a pair is due to chance alone. Standard errors and probabilities were calculated by the use of Student's "t" test

Experiment	Group	Description	No of stems	Hours for regeneration	Hours for movement	*p*	Hours for differentiation	*p*
I	A	Distal piece	19	22.6±0.3	14.1±0.2	≪0.001	8.5±0.2	≪0.001
	B	Proximal piece	19	27.9±0.4	21.6±0.2		6.3±0.3	
II	C	Short piece	6	68.3±7.9	32.0±4.4	≃0.1	36.3±6.5	≃0.4
	D	Long piece	16	53.8±3.7	24.2±2.1		29.6±3.0	
III	E	Narrow piece	16	48.5±4.6	22.5±2.2	≃0.005	26.0±4.0	≃0.4
	F	Wide piece	16	59.1±3.1	32.7±2.1		26.4±2.2	
IV	G	Distal end of piece ligated in middle	17	33.3±1.0	21.4±0.7	≪0.001	11.9±0.7	≃0.1
	H	Proximal end of stems in Group G	18	46.5±1.0	33.0±0.8		13.5±0.7	
V	I	See G	19	41.2±2.9	25.8±2.8	<0.001	15.4±0.8	>0.9
	J	See H	15	75.2±5.3	59.9±5.0		15.3±1.7	

being composed of pieces taken from the proximal region of long, unbranched stems, while the pieces of Group A are taken from the distal region. Thus in the case of distal versus proximal pieces of long stems, the slower rate of regeneration of the proximal pieces is entirely due to a slower rate of distad movement of the tissue, preceding the establishment of the hydranth primordium. This means that the axial gradient, as manifested by graded differences in rate of regeneration, is in reality a gradient in rate of tissue movement rather than a gradient in general "metabolic rate" prevailing during the entire period of regeneration. That the physiological processes occurring during the differentiation of a new hydranth

are certainly not slower in the more proximal regions is clearly shown by the fact that in these experiments the differentiation process itself is actually more rapid in proximal than in distal regions. In the other experiments, also, dealing with two groups regenerating at different rates, the difference in rate of regeneration between the two groups is in every case due to a difference in the time occupied by the tissue movement. In no case is it due to a difference in the rate of differentiation.

2. Reverse movement, rate of differentiation, and dedifferentiation

Table I shows that groups taken from different colonies may regenerate at markedly different rates. (Each experiment was performed with stems from a separate colony from which both groups within the experiment were taken.) Individuals *within* a colony may also regenerate at widely different rates. Table II illustrates the amount of variation in regeneration rate which may exist between comparable pieces from stems taken from a single colony. This particular sample

TABLE II

An example, drawn from the protocols of Group F, to show the amount of variation in rate of regeneration which may exist among comparable stems from the same colony. The figures indicate the number of stems in each stage at the times given

Time in hours	Nothing	Optical density gradient	Distal thickening	Proximal ridges	Distal ridges	Constricted or emerged
16.5	15	5	0	0	0	0
21.0	12	4	4	0	0	0
25.5	11	4	3	2	0	0
36.0	2	1	12	3	2	0
40.0	1	0	9	7	1	2
47.0	1	0	4	8	5	2
55.0	1	0	3	4	4	8
71.0	0	0	0	2	1	17

(Group F) was selected because it possessed the greatest amount of variation of any of the groups in these experiments. Not only does rate of regeneration considered as a whole vary, but the rate of differentiation also varies widely from colony to colony, as shown in Table I, and between different individuals from the same colony, as shown by the fairly sizeable standard errors for differentiation in some groups.

The protocols of the experiments show that neither the tissue movement nor the differentiation is always continuously progressive. In a large number of cases the tissue movement has been found to stop and then to reverse itself. In fact, regeneration at the proximal end of a piece ligated in the middle or of a long piece in which dominance does not act is dependent upon such a reversal in the direction of movement of the tissue (Steinberg, 1954). This is usually true in *T. crocea,* with which the present experiments were performed. However it is invariably true in the species (probably never identified) used for the experiments just referred to, because in that species the tissue movement is much more pronounced than it is in *T. crocea.* In yet a third species (referred to by Mr. Gray

of the Supply Department of the Marine Biological Laboratory as "the long, white kind") the movement is equally pronounced, while in a fourth species (referred to as "the short, brick-red kind," and probably *T. tenella*) the movement does not occur—a fact which has marked effects upon the phenomenon of dominance. In addition to reverses in the direction of movement, many cases have also been observed in which the differentiation process came to a standstill and then reversed. In the most extreme case a stem in the distal tentacle ridge stage, on the verge of constricting, began to dedifferentiate and continued this process until all traces of differentiation disappeared, including the thickening which had marked the primordium of the regenerating hydranth. At the same time it was observed that the tissue was moving back toward the proximal end of the piece. Much later a new primordium appeared and this time regeneration was completed. Dedifferentiation in *Tubularia* has been described before (Driesch, 1897, 1908; Peebles, 1900), but only as one possible aftermath to the cutting of the differentiating primordium. In the present cases it appears as a not infrequent occurrence in normal regeneration.

The observation that during the dedifferentiation of one primordium the tissue was undergoing a shift back toward the proximal end suggested that the many cases of dedifferentiation might be attributable to such movements. This seemed reasonable because (1) differentiation does not begin until a certain minimal concentration of cells has been accumulated at the presumptive regeneration site, and (2) the degree of regulation within a one- or two-mm. piece is also dependent upon cell number (Steinberg, 1954). This suggests that the continued differentiation of the primordium might be very sensitive to a decrease in its cell density, such as would occur if the tissue of the stem were to move away from it during the course of its differentiation. A direct test of this hypothesis by observation is not always possible because only rather gross movements of the tissue can be detected. However a rough test can be made by plotting the mean time for differentiation in a group against the number of reverses per stem in the movement phase. There are difficulties in the preparation of such a graph. It is not easy to decide in what cases a reversal in the direction of movement as noted in the protocols is truly a reversal and in what cases it merely reflects the fair amount of subjectivity involved in such observations. As has been mentioned before, in order to preserve as much objectivity as possible, the previous recordings for a regenerating stem were never looked at when a new recording was being made. As a result a considerable number of borderline cases are placed on one side of the line in one recording and on the other side in the next. Quite obviously these cases cannot be considered as true reverses in the movement of the tissue. Therefore the following three alternative criteria were established for the validity of reverse recordings. (1) Both the original stage and the subsequent reverse in the movement must be established by at least two consecutive similar recordings for each stage; (2) there must be at least two consecutive recordings which progress in the reverse direction; or (3) the recorded reverse in the movement must be of such magnitude that it could not be attributed to a difference in judgment. When these criteria are rigidly applied, the number of reverses is reduced so drastically that the maximum number in any group is four (Group D), with other groups yielding two reverses (Groups C, F and J), others yielding only one (Groups E, H and I), and the remaining groups yielding none (Groups A, B and G). These

criteria, although they are the best that could be devised, are for two reasons not nearly adequate for the establishment of an accurate correlation between reverse movements and the mean rate of differentiation. (1) In some groups there is a very large number of reverse recordings which do not meet any of the above criteria for significance, while in other groups there are none or very few. This

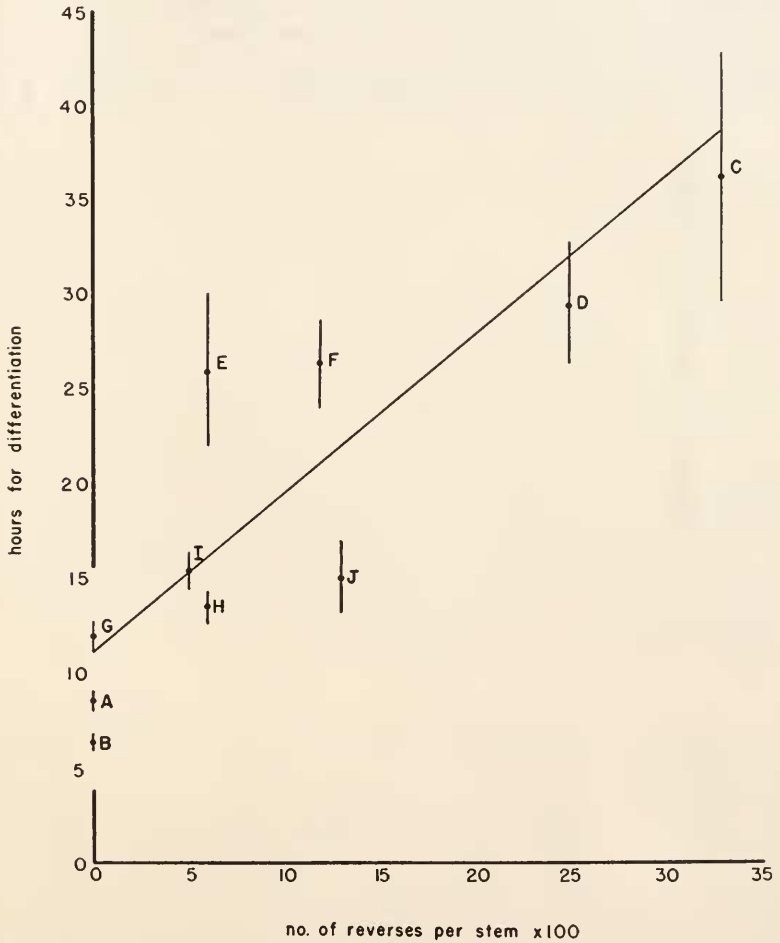

FIGURE 1. Correlation between the number of reverses during the movement phase and the time required for differentiation in the various groups. The line was arrived at by the Method of Least Squares.

can only mean that many reverse movements of short duration occur in some groups but cannot be distinguished from reverse recordings which are attributable to differences in judgment. (2) The number of reverses that meet one of the three criteria for significance is so small that it is bound to introduce large errors in the calculations. Nevertheless a very rough correlation between reverse move-

ments and rate of differentiation should be found if such movements really act to retard or reverse the differentiation process. That this rough correlation does indeed exist is shown in Figure 1. There is a general tendency for groups showing a higher percentage of reverses in the movement phase to show a slower mean rate of differentiation.

From the above we see that at least part of the differences in rate of differentiation which exist between colonies and between individual stems from the same colony are due to retardations and reversals in the differentiation caused by a movement of the tissue away from the differentiating primordium. The knowledge of such a correlation opens the way for a more quantitative treatment of the relationship between

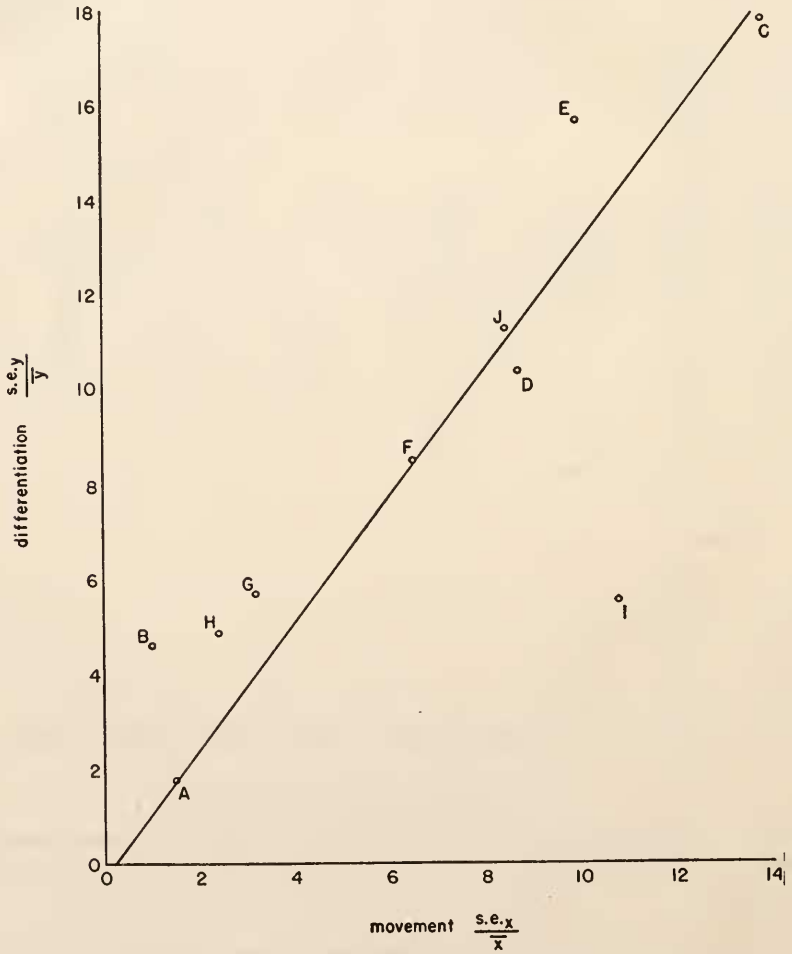

FIGURE 2. Correlation between the variation in time required for movement and the variation in time required for differentiation in the various groups. $\bar{x}$ = mean time for tissue movement; S.E.$_x$ = standard error of the mean for movement; $\bar{y}$ = mean time for differentiation; S.E.$_y$ = standard error of the mean for differentiation. The values on both axes are multiplied by 100. The line was arrived at by the Method of Least Squares.

variations in tissue movement and variations in rate of differentiation among similar stems from the *same* colony. The variation in tissue movement among the stems in a group can be numerically expressed by the figure $S.E._x/\bar{x}$, where $\bar{x}$ is the mean time for movement and $S.E._x$ is the standard error of the mean. Similarly the variation in rate of differentiation in a group can be expressed by $S.E._y/\bar{y}$, where $\bar{y}$ is the mean time for differentiation and $S.E._y$ is again the standard error of the mean. Since the variation in rate of differentiation has been shown to be caused, at least in part, by variations in tissue movement, the relationship between the two can be expressed by plotting the values of $S.E._x/\bar{x}$ against the values of $S.E._y/\bar{y}$ for the various groups and fitting the best possible straight line to the resulting points. This involves the assumption that the tendency of the stems in a group to undergo variations in movement is constant throughout the entire regeneration period. Should a particular group by chance show more variation in movement during one phase than during the other, the calculated value of $S.E._x/x$ for that group will be correspondingly high or low. Nevertheless, as far as can be determined, such fluctuations should occur equally in both directions, so that the over-all accuracy of the plotted line should not be seriously affected. Figure 2 shows the resulting graph. The fact that the line, obtained by the Method of Least Squares, passes almost directly through the origin indicates that differences in the movement of the tissue probably represent the *only* systemic factor contributing to variations in rate of differentiation among similar stems from the same colony. It does not, however, exclude the possibility that other factors may contribute to the large differences in rate of differentiation between *different* colonies. The slope of the line (1.35) indicates that the differentiating primordium is quite sensitive to slight movements of the tissue, an increase of 1 in $S.E._x/\bar{x}$ being responsible, on the average, for an increase of 1.35 in $S.E._y/\bar{y}$. This constitutes evidence that the differentiation process is continuously dependent upon a critical cell density in the primordium, and that when the cell density falls below this critical level the process of differentiation stops and even reverses.

3. The nature of the tissue movement

The movement of the tissue toward the distal end of the stem is due to an apparent contraction of the tissue as a whole in the distal part of the stem, accompanied by a stretching of the tissue in the proximal part (Steinberg, 1954). The result is the appearance of a gradient in optical density of the stem, the optical density being greatest distally, where the cells are the most numerous, and progressively decreasing proximally. It was considered likely, in view of a number of observations, that the tissue movement is of an amoeboid nature. Amoeboid movements of two ectodermal cell types of gymnoblasts, the cnidoblasts and the interstitial cells, have been described by Hadzi (1909), Kuchner (1934), Moore (1952), and Spratt (unpublished). The author has observed the amoeboid activity of the ectoderm of *Cordylophora, Eudendrium* and *Pennaria,* but has found no evidence of amoeboid movement of the endoderm. This situation seems to be reversed in *Hydra* (Papenfuss, 1934; Lehn, 1953), but seems to be rather general among the gymnoblasts.

In order to determine whether the distad movement of the tissue is of an amoeboid nature, the following experiment was performed. Twenty-five 7½-mm. stems were cut and all of the tissue was extruded from the perisarc with the ex-

ception of a band of tissue 1½ mm. long in the middle of the stem. If the movement of the tissue is of an amoeboid nature, the entire length of tissue should move toward the distal cut end of the perisarc. In 20 of the 25 stems the tissue did indeed move toward the distal cut end of the perisarc, traversing the three-mm. distance in the course of about 6 hours. In two stems the tissue moved in both directions, completely occupying the 7½-mm. length of perisarc in approximately the same length of time, while in only one case did the tissue fail completely to move. The tissue of the remaining two stems underwent incomplete distad movement. In many of the stems an optical density gradient appeared during the course of the movement. Two attempts were made to repeat this experiment, 5 stems being used each time, for the purpose of obtaining a photographic record of the movement, but each time the tissue moved toward both ends of the perisarc equally. The most likely explanation of this is that in both of the latter attempts colonies were used which had been in the laboratory for two or three days, such colonies giving aberrant results in many different types of experiments. An examination of the advancing edge of the tissue showed that it was composed of a very thin sheet of cells moving on the inner wall of the perisarc. At some points it could be seen that the extreme margin was a single layer of ectoderm cells, the endoderm terminating a few cells behind the advancing edge.

It is concluded that the movement of the tissue during the first phase of regeneration in *Tubularia* is an amoeboid movement of the ectoderm, the endoderm presumably being carried along passively because of its firm adhesion, by means of the mesoglea, to the ectoderm. Whether all of the cells of the ectoderm are amoeboid or whether the movement is due to the activity of certain specific cell types cannot be said at this time. It follows that the axial gradient in *Tubularia*, as manifested by graded differences in rate of regeneration, is a reflection of a gradient in the rate of movement of the amoeboid cells of the ectoderm.

Discussion

It is claimed by Tardent (1952, 1954) that the interstitial cells of hydroids are of great importance in the regeneration process and that the observed apico-basal gradient in the number of interstitial cells is the basis of the axial gradient in rate of regeneration. In the first paper it is stated that there is no longer any doubt that these cells migrate toward the cut surface where they form the "blastema." The author presents no observations to support his statement, relying upon claims to this effect in the literature. Perhaps the experiment most suggestive of such a phenomenon is that of Evlakhova (1946), in which the basal halves of normal individuals of *Hydra attenuata* were grafted to the apical halves of x-irradiated individuals of *Pelmatohydra oligactis* which are incapable of regeneration and tend to disintegrate. Regeneration followed amputation through the irradiated *Pelmatohydra*, the interstitial cells and cnidoblasts of the latter now being found to be those of *H. attenuata*. This, however, does not constitute evidence that the interstitial cells *formed* the regenerant. It is well known that the presence of non-irradiated tissue can vitiate the effects of x-irradiation elsewhere in the system, possibly by the contribution of -SH compounds (Barron, 1946; Jacobson *et al.*, 1949; Chapman *et al.*, 1949; Patt *et al.*, 1949; Abrams and Kaplan, 1951; Allen, 1951; Gershon-Cohen *et al.*, 1951). In his second paper Tardent acknowledges

(p. 628) that in *Tubularia* there is no such thing as a regeneration blastema and briefly considers the possibility that the other cells of the tissue may also contribute to the regenerant. However if one isolates a one-mm. length of stem, the entire piece transforms directly and *completely* into hydranth structures. This would appear to be a clear demonstration that the process of regeneration involves *all* of the cells of the regenerating region. The postulate that the axial gradient in rate of regeneration is due to a gradient of the cellular precursors of the regenerant is clearly inconsistent with the above observation.

The only existing theory up to the present time which attempts to explain the determination of the size of the hydranth primordium and the time required for regeneration is that of Barth (1944). This author points to the fact that both primordium size and the regeneration rate can be decreased by exposure to low oxygen tensions during the course of regeneration. He shows further that stems consume less oxygen at lower oxygen tensions and concludes that since energy production would be a function of oxygen consumption, both primordium size and regeneration rate are determined by the rate of energy production. In the light of the new facts concerning amoeboid movement of the tissue during regeneration and its importance in determining regeneration rate, a reinterpretation of Barth's data becomes possible.

In Barth's paper we read (p. 361), "During from 5 to 22 hours of exposure to N_2 sea water the stems appear to undergo no regeneration, since, when they are returned to O_2 sea water, they take the same time for regeneration as freshly cut stems." From this we can conclude that the amoeboid movement of the tissue cannot proceed anaerobically. Considering this, plus the fact that both primordium length and regeneration rate are determined during the movement phase of the regeneration process, it would appear that the action of low oxygen tension in reducing the primordium length and the regeneration rate is upon the amoeboid movement of the tissue, which would slow down and eventually stop as the oxygen tension is decreased. Barth also points to Peebles' finding (1931) that after 15 hours of regeneration the as yet unformed primordium could no longer be reduced in size by removing a large piece from the proximal end of the stem, that between 6 and 13 hours some of the primordia could be reduced in size, and that from two to four hours the size of all of the primordia could be reduced. He correlates this with his own finding that exposure to low oxygen tensions for various periods during the course of regeneration can decrease the size of the primordium, and concludes (p. 364) that "The results show that determination of the length of the primordium is progressive with time. There is no evidence of a sudden determination at some definite time. On the contrary, it is possible to change the length of the primordium during most of the period of regeneration. This implies that the distal parts of the hydranth are determined first and the more proximal parts later, for the changes in length of the primordium mean a change in the amount of stem derived from the proximal regions. Obviously, this proximal region forming the base of the hydranth must remain undetermined until the final few hours of regeneration." Since it has been found that the length of the primordium (and the rate of regeneration) is determined during the movement phase of regeneration and *some time before* the primordium is established, while determination of the parts of the regenerating hydranth cannot be detected until *after*

the primordium is established, it would seem more fruitful to consider these as two separate processes. If this is done, we can no longer consider that regional functional determination within the primordium proceeds basipetally or that the basal region of the hydranth remains undetermined until regeneration is nearly complete.

The statement that the length of the primordium can be changed during most of the period of regeneration is open to question and might best be discussed in connection with another experiment in Barth's paper. Stems were cut and divided into four groups. The first group, which served as a control, was allowed to regenerate under normal conditions. The second, third and fourth groups were treated with 0.07 *M* urethane for the periods from 0–10, 10–20, and 20–30 hours after cutting, respectively. The results show that regeneration in these groups was delayed 13.7 hours, 17.4 hours, and 4.4 hours, respectively, while the primordium length was also decreased, although not in proportion to the delay in regeneration. This latter fact is used as evidence that primordium length and regeneration rate are controlled by factors operating at different times. This conclusion is at variance with other findings already presented, but it might be pointed out in addition that when stems regenerate at low temperatures, regeneration rate is decreased (Moore, 1910; Moog, 1941) while primordium length is increased (Moog, 1941). It may be that at low temperatures the initiation of differentiation is delayed more than the tissue movement, so that more cells have a chance to enter the region in which reconstitution will occur before the differentiation begins, resulting in an increase in the size of the primordium. A similar effect may explain the lack of parallelism between regeneration rate and primordium length in Barth's urethane experiment.

An explanation is needed for the fact that Barth has reported an effect of low oxygen tension and of urethane on the length of the primordium well beyond the time at which both Peebles and the author have found the length to be no longer alterable by the removal of a large proximal piece of the stem. The following discussion may serve to bring these apparently contradictory findings into closer agreement. In Barth's experiments the stems were treated with urethane during specific intervals of time rather than during specific stages in their regeneration. Table II shows the wide spectrum of stages in which similar stems from the same colony may be at any given time. Admittedly the variation shown in Table II is the largest found in any of the groups in the present experiments, but the fact remains that there may be a considerable spread in regeneration rate among the stems of an experimental series. Treatment during specific intervals of time, then, may result in different effects upon different stems, depending upon their stage. This would not be revealed in Barth's data because he presents his results as averages. If there were a very specific stage at which the treatment is effective, the effect would be manifested as one which gradually appears and then gradually disappears, due to the use of averaged data and specific time intervals rather than specific stages. This is actually shown by the results. Finally, the time intervals during which the animals were treated are referred back to the time period for normal regeneration and used as a measure of the sensitivity of the controls to urethane treatment. However, if stems treated for the period between 10 and 20 hours after cutting are delayed an average of 17.4 hours, the treatment has not only stopped all regeneration at around 10 hours, but has even occasioned

the necessity of a mean recovery period of about 7.4 hours. It can be inferred from these results that the control stems were highly susceptible to the treatment at 10 hours, but no inference can be drawn concerning their susceptibility at, say, 15 or 20 hours because the treated stems did not pass through a comparable stage until some time after the cessation of the treatment. It would appear, then, that the effects of oxygen and of urethane upon regeneration rate and primordium length may be interpreted as having terminated at an earlier time than would at first be suspected. "Determination" of the length of the primordium would be expected to proceed gradually after the tissue movement has begun, and to terminate at the time when a certain critical concentration of cells has been reached at the presumptive regeneration site. It may be stated in summary that all existing evidence is consistent with the view that both the regeneration rate and the primordium length are determined during the phase of tissue movement and before the appearance of the primordium. One qualification should be added, however. We have seen that a reverse movement during the course of differentiation may slow down, stop, or even reverse the differentiation process. This being the case, the determination that we speak of is at best a labile one, and in the very strictest sense it would not be true to say that the regeneration rate of a particular stem is fixed when the primordium makes its appearance. This does not, of course, alter the fact that differences in regeneration rate between two *groups* of stems from the same colony, such as have been studied in the present experiments, are entirely due to differences in the time occupied by the movement (pre-differentiation) phase of the regeneration process.

There appears to be good evidence that there is a gradient in oxygen consumption, high distally and decreasing proximally, in the tubularian stem. Hyman (1926), Barth (1940), and Sze (1953), using a modified Winkler method, a Warburg respirometer, and the Cartesian diver method, respectively, have all found this to be true. Certain objections can be raised to these experiments, such as the number of approximations made by Hyman, the inclusion by Barth and Sze of the perisarc, which is thicker proximally, when taking dry weights, and the unusually small number of cases (14 stems divided among three experiments) in the experiments of Sze. Nevertheless, even allowing for these objections, it still appears that a respiratory gradient exists. If respiratory rate can be assumed to be a good measure of the physiological processes occurring, what explanation can be offered for the lack of an apico-basal gradient in rate of differentiation? In the present experiments a gradient in rate of differentiation has been found, but it goes in precisely the opposite direction. A possible explanation is to be found in the measurements made by Barth (1940) on oxygen consumption during the course of regeneration. This author finds (p. 369) that "The (respiratory) rate is highest from about 7 to 16 hours and it is during this period that the size of the primordium is determined (Peebles, '31). These changes in rate are observed only in the case of short (2–4 mm.) stems. The S-shape of the curve is lost or almost lost when the data from long (8–15 mm.) stems are plotted." It has been found (Steinberg, 1954, and the protocols of the present experiments) that in short stems the tissue moves toward the distal end and then ceases movement, and that after a hydranth regenerates distally it produces an inhibitor which prevents further movement. In long stems the tissue continues to move, even after the regeneration of a hydranth distally, presumably because of the dilution

of the inhibitor below an effective concentration by the increased volume of the coelenteric fluid. These observations correlate perfectly with the observations of Barth quoted above if one assumes that the differences in oxygen consumption which appear are measurements of the differences in the movement of the tissue. It has already been inferred that the tissue movement is a process which requires oxygen. Thus we see that oxygen consumption is higher distally than proximally, and the tissue moves more rapidly distally than proximally; oxygen consumption is highest in short stems from about 7 to 16 hours after cutting, and the tissue movement is occurring in short stems from the average colony at this very time; the above peak in oxygen consumption is not found in long stems, and the tissue does not stop moving in long stems. It would appear, then, that the gradient in oxygen consumption is in reality a measure of the rate of tissue movement in the different levels of the stem. However the question of what causes the cells from basal regions to move more slowly than those from apical regions must still remain a subject for future investigation.

Summary

1. The regeneration process in *Tubularia* is divisible into two phases. There is a period of tissue movement, during which extra cells are provided to the presumptive regeneration site, followed directly by the period of differentiation of the new hydranth.

2. Differences in rate of regeneration between narrow versus wide stems, the distal versus the proximal ends of stems ligated in the middle, and apical versus basal stem levels are all due to differences in the time occupied by the tissue movement.

3. Narrow and wide stems differentiate at the same rate, as do the distal and proximal ends of stems ligated in the middle. However, pieces from *basal* stem levels differentiate, in these experiments, at a significantly *greater* rate than pieces from *apical* stem levels.

4. Both rate of regeneration considered as a whole and rate of differentiation vary from colony to colony and between comparable stems taken from the same colony.

5. Neither the tissue movement nor the process of differentiation is always continuously progressive. Reverses in the direction of movement and spontaneous dedifferentiation of the differentiating primordium have both been observed.

6. The differentiation process is continuously dependent upon a critical cell density in the primordium. Evidence is presented that differences between stems in the movement of the tissue during the differentiation process represent the only systemic factor responsible for variations in differentiation rate among similar stems from the same colony.

7. The tissue movement is due to the movement of the amoeboid cells of the ectoderm, and the axial gradient, as manifested by an apico-basal decrease in rate of regeneration, is a reflection of the apico-basal gradient in the rate of tissue movement.

8. Both the apico-basal gradient in oxygen consumption and differences in oxygen consumption during regeneration correlate perfectly with the rates of tissue movement at different levels of the stem and with the period during which

the movement occurs. It is inferred that differences in oxygen consumption indirectly measure the differences in tissue movement, which is an aerobic process.

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